DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/18/2010 has been entered.

2. Claims 29 and 47 were amended. New claims 59-60 have been added. Accordingly, claims 29, 31-38, 47, and 57-60 are currently pending and subject to examination below in light of the elected species of amino acids 32-48 of canine proBNP (SEQ ID NO:3) as the species of proBNP epitope.

Objections/ Rejections Withdrawn

3. The objections to the drawings have been withdrawn in view of the replacement drawings filed on 5/18/2010.

4. The objections to the specification and claims have been withdrawn in response to Applicant's amendments thereto.

Priority

5. Acknowledgment is made of the present application as a proper National Stage (371) entry of PCT Application No. PCT/EP05/54446, filed 9/8/2005, which claims foreign priority under 35 U.S.C. 119(a)-(d) to Application No. A 1505/2004, filed on 9/8/2004 in Austria.

Claim Rejections - 35 USC § 112

6. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 29, 31-38, 47, and 57-60 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The nature of the invention relates to an immunoassay method for detecting canine proBNP or a fragment thereof in a canine blood or urine sample. The methods uses an antibody that binds an epitope within the amino terminus of canine proBNP, in particular the elected species of an epitope in the region from amino acids 32 to 48 of the protein (see especially claims 29, 31, 47 and 57). It appears that Applicant intends this epitope to refer to the last sequence depicted in Figure 1B (SEQ ID NO:3).

Applicant has provided evidentiary testimony that, as of 2004, it was unknown how many forms of BNP existed in tissue and in blood and that further, it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies. In particular, Applicant declares that it was not possible at the time of invention to predict that any circulating form of BNP would also present and be able to be detected using antibodies in dogs. Applicant further declares that it was unclear at the time of invention which of the various BNP forms would be

useful as markers of pathophysiologic processes. See the Declaration of Dr. Boswood filed 5/18/2010, in particular at items 4-7, 14-15, and 18-20.

For example, Dr. Boswood declares that as of 2004 it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment thereof (item 5). In items 19-20, Dr. Boswood further declares that:

> More specifically, the following were neither known nor could be predicted: (1) how many forms of BNP were present in the tissues and blood of humans and other species; (2) which forms of circulating BNP would be predominant in species such as cats and dogs; (3) whether any forms of circulating BNP would be stable enough to be detected by antibodies in cats or dogs; and (4) which forms of BNP would be useful as markers of pathophysiologic processes.
>
> For the foregoing reasons, as of September 8, 2004, it was unknown whether circulating proBNP could be detected in dogs or cats. Furthermore, it was not possible to predict that antibodies directed against a particular amino acid region of dog proBNP could detect circulating proBNP in dogs. Likewise, it was not possible to predict that antibodies directed against a particular amino acid region of cat proBNP could detect circulating proBNP in cats.

Dr. Boswood further declares that that "it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment of proBNP" and similarly that "it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies" (see items 5 and 7, respectively).

Such statements by Applicant indicate that at the time of invention, it was not possible to predict whether fragments of canine proBNP containing exist in canine blood and urine, and that in particular, it was not possible to predict whether fragments containing epitopes found within amino acids 32 to 48 of canine proBNP would exist in these samples. Further, Applicant's remarks indicate that it was not possible to predict whether such fragments would provide informative clinical information about the presence of disease.

If the existence of canine proBNP or fragments thereof in canine blood or urine could indeed not be predicted as argued by Applicant, this means that one of ordinary skill in the art would not envisage possession of the claimed methods without verification that such species actually exist and are correlated with disease.

The instant specification discloses experiments in which antibodies raised against amino acids 25-41 and 74-86 of canine proBNP were used to analyze samples from healthy and sick dogs. See Example 3 and Figure 3A in particular. However, the instant specification fails to disclose experiments in which antibodies were raised against amino acids 32 to 48 of canine proBNP.

Consequently, the data presented in the specification do not verify the existence of canine proBNP or fragments thereof that contain epitopes within amino acids 32 to 48 of canine proBNP. Further, the data presented in the specification do not document a correlation between elevated levels of such species in blood or urine with cardiac insufficiency in dogs.

In summary, Applicant has taken the position in the instant Reply that it was not possible to predict *a priori* whether antibodies binding to particular region of canine proBNP could be used to detect proBNP in blood or urine. If adopted, such arguments and evidence mean that it

would not be possible to predict whether an antibody binding to an epitope within amino acids 32 to 48 of canine proBNP could be used to detect canine proBNP or fragments thereof in the blood or urine of dogs. Yet the instant specification adds nothing further to this inquiry, as such an antibody was not actually made.

Similarly, if it is true as Applicants argue that the existence of proBNP or fragments thereof that are detectable via the claimed antibody could not have been predicted at the time of invention, and further that it could not be predicted whether such fragments were stable enough to be detected, then this means that actual data or other means of verifying their existence would be necessary in order to envisage possession of the claimed invention. In addition, if Applicant's evidence and arguments that it was not possible to predict which forms of BNP would be useful as markers of pathophysiological processes are adopted, this means that data regarding BNP forms that are detectable by the claimed antibodies and their correlation with cardiac insufficiency would be necessary. Yet the instant specification adds nothing further to this inquiry, as such data documenting the existence of the BNP species at issue and their correlation with disease are also lacking in the instant specification.

8. Claims 29, 31-38, 47, and 57-60 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The nature of the invention is as discussed in detail above. As also discussed above, Applicant has submitted evidence and arguments regarding the unpredictability in the art.

Specifically, the Boswood Declaration filed 5/24/2010 declares that that "it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment of proBNP" and similarly that "it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies" (see items 5 and 7, respectively).

The Boswood Declaration also includes the following testimony regarding the state of the prior art and the unpredictability of the art:

> More specifically, the following were neither known nor could be predicted: (1) how many forms of BNP were present in the tissues and blood of humans and other species; (2) which forms of circulating BNP would be predominant in species such as cats and dogs; (3) whether any forms of circulating BNP would be stable enough to be detected by antibodies in cats or dogs; and (4) which forms of BNP would be useful as markers of pathophysiologic processes.
>
> For the foregoing reasons, as of September 8, 2004, it was unknown whether circulating proBNP could be detected in dogs or cats. Furthermore, it was not possible to predict that antibodies directed against a particular amino acid region of dog proBNP could detect circulating proBNP in dogs. Likewise, it was not possible to predict that antibodies directed against a particular amino acid region of cat proBNP could detect circulating proBNP in cats.

The claimed invention is directed to a method of detecting canine proBNP or a fragment thereof in a canine blood or urine sample, using an antibody that binds an epitope within the region from amino acids 32 to 48 of canine proBNP.

However, the instant specification does not provide a working example of such an antibody. As such, if Applicant's arguments and evidence regarding the unpredictability in the art are adopted, the instant specification is not enabling because it adds nothing further to the prior art. In particular, if it is true as Applicants have argued that it cannot be predicted whether an antibody against a particular amino acid region of proBNP could be made, or similarly which amino acid regions of the various forms of BNP could be detected using antibodies, then the logical consequence of such reasoning is that actual data would be needed. In other words, if Applicant's arguments and evidence are adopted as true, it would remain unpredictable whether antibodies against amino acids 32 to 48 of canine proBNP could be made, since the specification fails to report that such antibodies were actually successfully made.

Similarly, if it is true as Applicants have argued that it was not possible to predict which forms of circulating BNP would be predominant in dogs and whether any such forms would be stable enough to be detected, then this uncertainty would still persist as the specification fails to document the existence and detection of circulating BNP forms that contain epitopes in the region from amino acids 32 to 48 of canine proBNP.

In addition, the preamble of claim 29 recites "[a] method of determining the presence and/or concentration of canine proBNP or fragments thereof". However, the claimed methods are insufficient to detect *any* fragments of canine proBNP as claimed. The epitope bound by the antibody is present within the amino-terminal portion of proBNP and not within the carboxy-terminal portion that corresponds to mature BNP. Consequently, the antibody used for the assay would not bind to BNP or to other fragments that lack the SEQ ID NO:3 epitope.

Furthermore, Applicant has argued on the record for the existence of unpredictable factors, stating that it was not previously known which fragments of canine proBNP circulated in blood or whether the amount of such fragments was sufficient to be detected by immunoassay. When taken together with such statements, the specification fails to predictably enable one of ordinary skill in the art to use the claimed methods to determine any fragment of proBNP as claimed.

In summary, Applicant has argued and advanced evidence on the record to the effect that the claimed invention was wholly unpredictable at the time of the invention. However, the specification fails to establish that antibodies that bind in the region from amino acids 32 to 48 of canine proBNP can be made and/or used in detection as claimed, as no data regarding such antibodies are reported therein. If Applicant's arguments of unpredictability are adopted, then it would require undue experimentation to practice the claimed invention as the specification fails to remedy this unpredictability.

9. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 29, 32-38, 47, and 58-60 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

11. Claims 29 and 47 recite "amino acids 20 to 86 of canine proBNP". The specification apparently discloses partial sequence information for the canine proBNP in Figure 1B, which lists for example the amino acid sequence corresponding to amino acids 32 to 48 of canine

proBNP as Epitope 3 (SEQ ID NO:3). It is presumed that the elected species of "amino acids 32 to 48" corresponds to SEQ ID NO:3.

However, the full amino acid sequence of canine proBNP is not disclosed in the specification. In addition, the sequence corresponding to "amino acids 20 to 86 of canine proBNP" is neither depicted in the Figures nor disclosed elsewhere in the specification. There is not enough information in the specification to uniquely identify "amino acids 20 to 86 of canine proBNP".

The specification indicates that the amino acid sequence for canine BNP has been published in the Swiss-Prot database under the accession No. P16859 (see page 4).

However, discrepancies were noted upon comparison of the partial sequence information listed in Figure 1B of the specification with the sequence published as Swiss-Prot accession No. P16859 (see the information as retrieved from the UniProtKP/Swiss-Prot database, http://www.uniprot.org/uniprot/P16859, on 3/10/2009).

For example, the amino acids numbered as "amino acids 32 to 48" of canine proBNP in Figure 1B of the specification do not correspond to amino acids 32 to 48 of accession No. P16859 (see the attached information for P16859 at the top of page 3). It appears that Applicant is employing a different numbering scheme. This raises ambiguity as to what specific amino acid sequences are intended. Consequently, the reference in the claims to particular amino acid sequences of canine proBNP by amino acid numbers alone is insufficient to uniquely identify the intended sequences.

In addition, other researchers have reported sequence information for canine proBNP that differs from the P16859 sequence. In particular, Seilhamer et al. (U.S. 6,586,396 B1, of record)

depicts a dog preproBNP sequence in Figure 8 that is not the same as the P16859 sequence (see the third row of Figure 8 where the sequence departs for several residues).

In summary, the specification does not provide enough information to adequately identify the recited amino acid sequences. Although reference is made to amino acid sequence information in a public database, the database sequence conflicts with the partial amino acid sequence information disclosed in the present application. In addition, the specification has not effectively incorporated by reference the database amino acid sequence information. Furthermore, it appears that differing amino acid sequence information was reported for canine proBNP. For all of these reasons, the metes and bounds of the claims are unclear.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 29, 31-33, 37-38, 47, and 57-59 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. ("Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure" J Vet Intern Med. 2003 Mar-Apr;17(2):172-7) in view of Asada et al. (EP 1 016 867 B1, of record) and in light of the evidence of Harlow & Lane ("Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, pages 23-24 and 76), the Academic Press Dictionary of Science and Technology (definition for the term "polyclonal"; Oxford: Elsevier Science & Technology (1996); retrieved

October 22, 2008, from http://www.credoreference.com/entry/3144515/), Janeway et al. (Immunobiology: the Immune System in Health and Disease (1999), Elsevier Science Ltd/Garland Publishing, New York, NY, Fourth Edition, pages 34-35), and Wolfe (Wolfe, S.L., Molecular and Cellular Biology, 1993, pages 790-793).

MacDonald et al. teaches that brain natriuretic peptide (BNP) is a recognized biomarker of cardiac disease and congestive heart failure in humans (the abstract). The authors performed clinical studies on dogs, in order to assess whether BNP is also a biomarker of canine heart disease. In particular, MacDonald et al. measured canine BNP levels in plasma samples from normal dogs and from dogs with heart disease or heart failure, and observed a significant positive correlation with disease (ibid and pages 174-176, "Discussion"). In addition, BNP levels increased with increasing severity of disease (ibid and pages 173-174, "Results"). MacDonald et al. conclude from these studies that increases in BNP may be used to predict death due to cardiovascular disease in dogs, much like in human medicine (see page 175, last paragraph to page 176, second paragraph).

The teachings of MacDonald et al. indicate that BNP is indicative of disease not only in humans, but also in dogs.

It is noted that MacDonald et al. measured BNP-32 (which is a fragment of proBNP corresponding to the carboxy-terminal 32 amino acids of proBNP which is released upon proteolytic cleavage) by radioimmunoassay (page 173, left column), but do not provide details regarding the measurement.

The teachings of MacDonald et al. differ from the claimed invention in that while the reference determined the concentration of the BNP-32 fragment of canine proBNP by

immunoassay, the reference fails to apparently teach the use of an antibody that binds to an epitope within amino acids 20 to 86 of canine proBNP.

Asada et al. teach that BNP-32 (also referred to in the reference as α-BNP or simply BNP) is first synthesized as the preprohormone prepro-BNP [0002]. This preprohormone includes a signal sequence which is cleaved to give pro-BNP (also referred to in the reference as γ-BNP) (ibid and [0010]). In the case of humans, pro-BNP has a total of 108 amino acids [0011]. Pro-BNP is then further split to produce BNP-32 and BNP(1-76). The latter fragment is also referred to in the reference as γ-BNP(1-76). BNP-32 is the carboxy-terminal fragment of proBNP, while BNP(1-76) is the amino-terminal fragment of proBNP [0007].

Asada et al. teach in blood, BNP exists in the form of proBNP or its degradation product, and not in the form of BNP-32 which was previously considered dominant [0008]. Asada et al. further teach that pro-BNP is more stable than BNP-32 in blood (ibid). Due to these facts, Asada et al. concluded that it is indispensable to assay not only BNP-32 but also pro-BNP in order to accurately diagnose cardiac disease.

To accomplish this, Asada et al. teach an immunoassay using a first antibody which is reactive with BNP-32 and a second antibody which is reactive with mammalian prepro-BNP or pro-BNP derivatives but not with BNP-32 [0009], [0012], [0017]. Note that Asada employ the term "derivatives" to encompass pro-BNP itself [0013].

The immunoassay preferably measures the pro-BNP derivative that corresponds to amino acid Nos. 27-134 of SEQ ID NO:1, in the case of human BNP [0019]. This sequence refers to pro-BNP, i.e. after cleavage of the 26-amino acid N-terminal signal sequence from prepro-BNP.

The second antibody used for the assay is preferably specific for amino acids 27-102 of SEQ ID NO:1 in the case of the human sequence [0019]. This sequence corresponds to BNP(1-76), i.e., pro-BNP after the carboxy-terminal BNP-32 has been removed. As one example, Asada et al. raised an antibody against amino acid Nos. 27-64 of SEQ ID NO:1 (i.e., amino acids 1-38 of human pro-BNP). See Example 1, in particular at page 5, lines 24-32.

Although the Example of Asada et al. involved antibodies against human BNP sequences, the reference clearly contemplates any mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1).

In light of the teachings of Asada et al., it would have been obvious to one of ordinary skill in the art to modify the teachings of MacDonald et al. so as to detect not only canine BNP-32 but also canine pro-BNP. In particular, the teachings of Asada et al. indicate that contrary to what was previously thought, BNP exists predominantly in the blood not as BNP-32 but as proBNP or other fragments thereof. In addition, Asada et al. taught that proBNP is more stable in blood than BNP-32. Consequently, Asada et al. teach the need to employ an assay that detects not only BNP-32 but also pro-BNP in order to accurately assess cardiac disease.

As such, when assessing cardiac disease in dogs based on BNP levels according to the method of MacDonald et al., one of ordinary skill in the art would have been motivated to employ this known variation by performing an immunoassay that detects not only canine BNP-32 but also canine pro-BNP in order to obtain more accurate clinical results in dogs in the same way that Asada et al. taught in the case of humans.

Put another way, because Asada et al. teach that their immunoassay produces more accurate clinical results than assays for BNP alone, it would have been obvious to adapt this

immunoassay in order to detect pro-BNP and BNP in any mammalian species; when taken together with the teachings of MacDonald et al. that BNP was also well established to be biomarker of heart failure in dogs, it would have been obvious to adapt the immunoassay format of Asada et al. to detect canine pro-BNP and BNP for the purpose of clinical assessment of heart failure in dogs.

The particular solution taught by Asada et al. for performing such an improved BNP immunoassay employs two antibodies, one specific for the carboxy-terminal fragment of pro-BNP (BNP-32) and one specific for the amino-terminal fragment of pro-BNP (which is BNP(1-76) in the case of human).

The Examiner notes that both human and canine BNP-32 are peptides of 32 amino acids (Asada et al., [0002]). However, the precursor pro-BNP sequences vary slightly depending on species (Asada et al., [0010]). This is why when Asada et al. refer to particular amino acid sequences or residues, the species is also specified (e.g., "In case of human [pro-BNP], it is pro-BNP of 108 amino acids" [0011]).

Therefore, when Asada et al. teach that the second antibody is preferably specific to BNP(1-76) or amino acids 27-102 of SEQ ID NO:1, one of ordinary skill in the art would readily understand that this numbering is referring to the amino-terminal fragment of *human* prepro-BNP (i.e., amino acids 1-76 of proBNP after removal of the 26-residue signal sequence). However, because Asada et al. clearly contemplates *any* mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1), it would have been obvious when performing assays for dog pro-BNP to employ a second antibody that is specific for the amino-terminal fragment of canine pro-BNP. Furthermore, Asada et al. also indicate that like human

proBNP, canine proBNP is also cleaved into amino-terminal and carboxy-terminal fragments, although this occurs at Arg 100 rather than Arg 102 due to slight differences in the sequences between species [0010].

When taken together, therefore, it would have been obvious to adapt the two-antibody assay of Asada et al. for detection of canine pro-BNP and BNP by employing a first canine BNP-32-specific antibody together with a second antibody specific for the amino-terminal fragment of canine proBNP which is released upon cleavage at Arg 102 of canine proBNP. One would be motivated to use two antibodies specific for both the amino-terminal and carboxy-terminal fragments of proBNP in this manner in order to detect not only BNP-32 but also proBNP and its derivatives, which leads to more accurate clinical results as taught by Asada et al.

As discussed above, it is not entirely clear what amino acid sequence is intended by "amino acids 20 to 86 of canine proBNP". However, the instant specification discloses that antibodies against the epitopes in these regions bind to canine NT-proBNP, which is the amino terminal fragment of proBNP or BNP(1-76) in humans (see the specification at page 13). As best understood, therefore, Applicant intends that the antibody binds to canine proBNP within the region that corresponds to the amino-terminal portion of canine proBNP (and not to the portion corresponding to BNP-32).

Therefore, when the claims are given their broadest reasonable interpretation second antibody suggested by the combination of MacDonald et al. and Asada et al. possesses the requisite specificity as it binds to the amino-terminal fragment of canine proBNP (i.e., the canine counterpart of BNP(1-76) in humans).

In addition, no evidence of criticality for the currently claimed range is apparent. Because the teachings of Asada et al. indicate that the region to which antibodies bind on preproBNP influence what fragments of this molecule may be detected, it would have been obvious to arrive at the claimed invention out of the course of routine optimization.

Similarly, with respect to claims 31, 47, and 57-58, although the references do not specifically teach the recited epitope consisting of amino acids 32 to 48 of canine proBNP, absent evidence of criticality it would have been obvious to arrive at the claimed invention out of the course of routine optimization.

It is also noted that Asada et al. teach that the antibodies may be either monoclonal or polyclonal [0018].

Polyclonal antiserum was well known in the art to comprise a mixture of antibodies of different specificities directed toward multiple antigenic determinants present on a particular antigen. See the Academic Press Dictionary of Science and Technology, which defines a polyclonal antibody as a population of heterogeneous antibodies derived from multiple clones, each of which is specific for one of a number of determinants found on an antigen. See also Janeway et al., which provides evidence that antibodies in serum (i.e., antisera) are polyclonal in nature, containing many different antibody molecules that bind to an antigen in many different ways (see p. 34-35, especially at p. 35, the second full paragraph, and Figure 2.1).

It was also well known in the art at the time of the invention that antibodies do not contact the entire surface of their target antigen but rather bind relatively small regions or "epitopes" within said antigen. See Harlow & Lane at pages 23-24, the section entitled "The

region of an antigen that binds to an antibody is called an epitope". Harlow et al. also provide evidence that peptide epitopes recognized by antibodies are generally only six amino acids in length, with some researchers reporting epitopes of even smaller size that can be successfully bound by an antibody (see page 76, the first sentence of the section titled "Size of the Peptide"). Similarly, Wolfe discloses that the size of an epitope bound by an antibody is between 3 to 16 amino acids in length (see particularly the bottom of the left column of page 791).

In light of the evidence of Harlow et al. and Wolfe, it is apparent that the recited amino acid sequences possess multiple epitopes available for antibody binding.

Furthermore, in light of the evidence of the Academic Press Dictionary of Science and Technology and Janeway et al., it is apparent that the polyclonal antibodies of Asada et al. would necessarily constitute a homogeneous population of antibodies that bind to the immunogens in many different ways, i.e. to different epitopes within this peptide.

As such, an especially absent evidence to the contrary, it appears that the polyclonal antibodies specific to the amino-terminal fragment of canine proBNP as suggested by the combination of MacDonald et al. and Asada et al. would necessarily include antibodies capable of binding to proBNP at an epitope within amino acids 20 to 86 or 32 to 48 of canine proBNP, given that these sequences share multiple epitopes in common with the amino-terminal fragment of canine proBNP.

Therefore, it would also have been obvious to arrive at the claimed invention of claims 29, 31-38, 47 and 57 by employing antibodies as above that are polyclonal, as taught by Asada et al. As a result of the nature of such antibodies as well as the relationship of the recite epitopes to

the amino-terminal fragment of canine proBNP, it appears that the resulting polyclonal antibodies would necessarily possess the recited binding characteristics.

With respect to claim 32, Wolfe (discussed above) provides evidence that antibody epitopes are 3 to 16 amino acids in length. Therefore, antibodies to canine proBNP according to the method of MacDonald et al. and Asada et al. would necessarily possess the recited characteristics.

With respect to claim 37, in the immunoassay of Asada et al., at least one of the first and second antibodies may be immobilized on a solid support [0021]. Therefore, when performing the method of MacDonald et al. using two pro-BNP-specific antibodies as taught by Asada et al., it would have been obvious to employ this solid phase-based assay format suggested by Asada et al.

With respect to claim 38, the immunoassay of MacDonald et al. and Asada et al. involves antibody binding and would therefore be considered an "immune binding assay".

With respect to claim 58, Asada et al. teach that the antibodies may be either monoclonal or polyclonal [0018].

14. Claims 34-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. in view of Asada et al. and in light of the evidence of Harlow & Lane, the Academic Press Dictionary of Science and Technology, Janeway et al., and Wolfe as applied to claim 29 above, and further in view of Harlow & Lane ("Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, pages 319, 321-323, 342-345, 353, 561, 563, 574,

and 591-593; hereinafter, "Harlow & Lane **2**"). See Applicant's Information Disclosure statement filed 3/27/2009.

MacDonald et al. and Asada et al. are as discussed in detail above. MacDonald et al. teaches radiolabeling (page 173, left column) but does not provide details. Asada et al. exemplifies directly labeling one of the antibodies used for the two-antibody assay (Example 1), and therefore fails to specifically teach the use of at least one further antibody that binds to the antibody discussed in detail above.

Harlow & Lane **2** teach that an antibody can be directly labeled or alternatively used with a labeled secondary reagent that will specifically recognize the antibody (see entire selection, especially at pages 321, 561 and 563). Such a secondary reagent may be (for example) a labeled anti-immunoglobulin antibody specific for the first antibody (see in particular pages 321-323, 345, 574 and 591-593). Detection labels may be, e.g. iodine, fluorochromes, enzymes such as peroxidase, or biotin (p. 342-344, 353, 561, 578-580, 591-593, and 320-323). The choice of direct versus indirect detection depends on the circumstances of the experiment; however, the use of directly labeled antibodies is less sensitive than indirect methods (page 321). In addition, direct labeling requires a new labeling step for every antibody to be studied. In contrast, indirect methods offer the advantages of widely available labeled reagents, which are commercially available and which can be used to detect a large range of antigens. Finally, with indirect labeling the primary antibody is not modified, so potential loss of activity is avoided. Consequently, for the majority of applications indirect methods are the most useful.

Therefore, it would have been further obvious to add a further secondary antibody specific for the proBNP-specific antibody in labeled form in the method of MacDonald et al. and

Asada et al. In particular, it would have been obvious to substitute indirect labeling in this manner for the direct labeling methods exemplified by Asada et al. because Harlow & Lane **2** taught for most applications, indirect methods are the most useful and offer numerous advantages over direct labeling.

With respect to claim 36, both MacDonald et al. and Asada et al. teach the use of radiolabels (MacDonald et al. at page 173, left column; radiolabeled iodine is taught by Asada et al. at page 5). Asada et al. also teaches enzymes, fluorescent substances, particles, etc. Similarly, Harlow & Lane that detection labels may be, e.g. iodine, fluorochromes, enzymes such as peroxidase, etc.

Therefore, when employing indirect labeling to label the secondary antibody as discussed above, it would have been further obvious to employ such known labels for their known purpose.

15. Claim 60 is rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. in view of Asada et al. and in light of the evidence of Harlow & Lane, the Academic Press Dictionary of Science and Technology, Janeway et al., and Wolfe as applied to claim 29 above, and further in view of Hrubec et al. ("Plasma Versus Serum: Specific Differences in Biochemical Analyte Values" Journal of Avian Medicine and Surgery 16(2):101-105, 2002).

The references are as discussed in detail above, which fail to specifically teach analysis of **serum** samples. In particular, MacDonald et al. and Asada et al. suggest plasma samples but not serum samples.

Hrubec et al. teach that biochemical analysis can be conducted using either plasma or serum, and that these are similar sample types (page 101, right column). The reference teaches

that the different sample types have different handling requirements in that plasma samples are kept on ice and centrifuged immediately, while serum samples can be allowed to clot at room temperature before centrifuging (the abstract and page 103, "Discussion").

Therefore, one skilled in the art would find it obvious to detect canine pro-BNP in serum rather than in plasma for convenience, in order to avoid having to immediately cool and centrifuge the collected sample. One would have a reasonable expectation of success given that Hrubec et al. teach that serum and plasma are similar samples differing only in that plasma contains clotting factors and an anticoagulant that are not present in serum. Thus, since natriuretic peptides such as proBNP are neither clotting factors nor anticoagulants, one skilled in the art would have a reasonable expectation of success in detecting canine pro-BNP in serum as well as plasma.

16. Claims 29, 31-38, 47, and 57-60 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. ("Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure" J Vet Intern Med. 2003 Mar-Apr;17(2):172-7) in view of Karl et al. (U.S. 2007/0059767 A1) and Liu et al. ("Cloning and characterization of feline brain natriuretic peptide" Gene 292 (2002) 183–190).

MacDonald et al. teaches that brain natriuretic peptide (BNP) is a recognized biomarker of cardiac disease and congestive heart failure in humans (the abstract). The authors performed clinical studies on dogs, in order to assess whether BNP is also a biomarker of canine heart disease. In particular, MacDonald et al. measured canine BNP levels in plasma samples from normal dogs and from dogs with heart disease or heart failure, and observed a significant positive

correlation with disease (ibid and pages 174-176, "Discussion"). In addition, BNP levels increased with increasing severity of disease (ibid and pages 173-174, "Results"). MacDonald et al. conclude from these studies that increases in BNP may be used to predict death due to cardiovascular disease in dogs, much like in human medicine (see page 175, last paragraph to page 176, second paragraph). The teachings of MacDonald et al. indicate a nexus between BNP and cardiac disease not only in humans, but also in dogs.

It is noted that MacDonald et al. measured BNP-32 (which is a fragment of proBNP corresponding to the carboxy-terminal 32 amino acids of proBNP which is released upon proteolytic cleavage) by radioimmunoassay (page 173, left column), but do not provide further details regarding the measurement.

The teachings of MacDonald et al. differ from the claimed invention in that while the reference determined the concentration of the BNP-32 fragment of canine proBNP by immunoassay, the reference fails to apparently teach the use of an antibody that binds to an epitope within amino acids 32 to 48 of canine proBNP.

Karl et al. discuss how human BNP is expressed as the 108-residue precursor proBNP, which is cleaved into N-terminal proBNP (NT-proBNP; amino acids 1-76 of proBNP) and the BNP (amino acids 77-108 of proBNP, also known in the art as BNP-32). See [0007].

Karl et al. teach that because BNP itself is not very stable, its use as a diagnostic marker is limited [0006]. As an alternative to BNP, Karl et al. teach determining NT-proBNP in a sample using two antibodies that detect different epitopes of this protein fragment, such that they are capable of simultaneously binding (the abstract; [0001]; [0011]-[0025], [0046]; and the claims). The antibodies specifically bind epitopes within the 76-amino acid NT-proBNP

molecule, preferably in the amino acid region from 10 to 66, and particularly preferred in the region 10 to 50 or 10 to 38 [0044]. The antibodies may be either polyclonal or monoclonal [0025], and may be raised using recombinant NT-proBNP as an immunogen [0043].

In the Examples, Karl et al. raised both polyclonal and monoclonal antibodies against recombinant human NT-proBNP and subsequently screened them to determine the most reactive epitopes. Antibodies which were reactive with amino acids 30-38 of proBNP (ELQVEQTSL; SEQ ID NO:8) were successfully produced in this manner (Example 2, see especially at [0062]; and Example 3, see especially Table 2, monoclonal antibody 13.1.18; and Table 3, polyclonal antibody S-9212).

The polyclonal antibody against amino acids 30-38 of human NT-proBNP was used together with a second polyclonal antibody against amino acids 1-21 of this molecule in an immunoassay for determination of NT-proBNP (Examples 4-6). Such assays can be used diagnostically to differentiate between healthy and heart failure patients (Example 6 and claims 28-39).

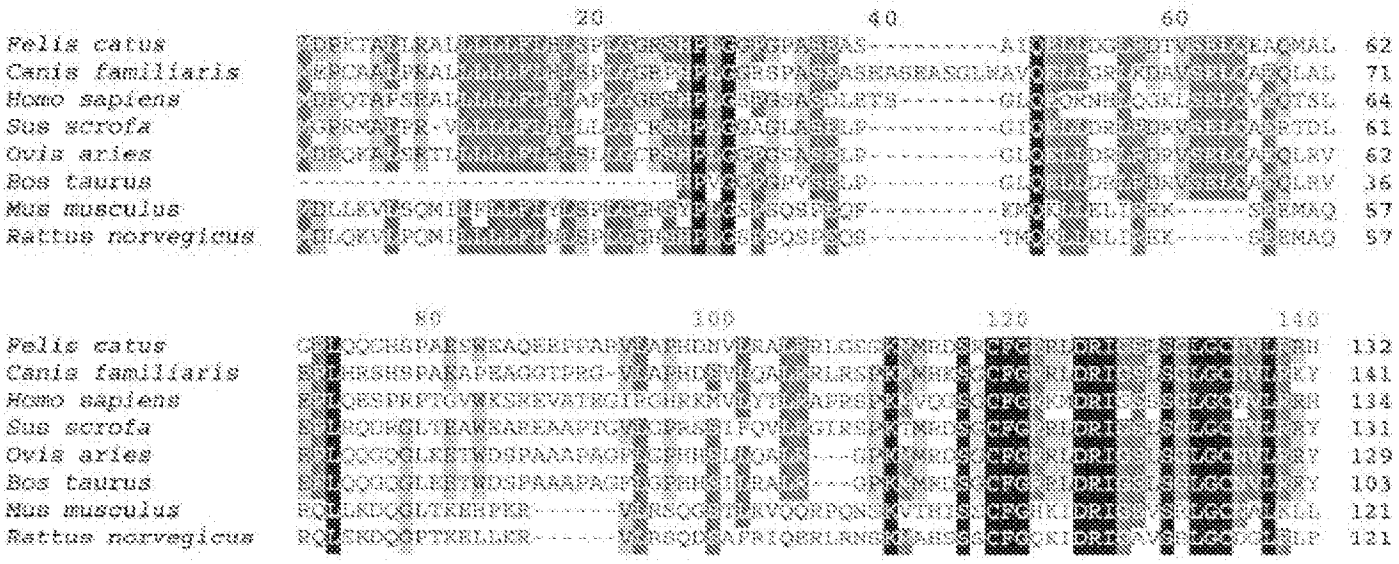
In summary, Karl et al. teach that like BNP, NT-proBNP is also a diagnostic marker in heart failure, but that NT-proBNP is more stable than BNP. When taken together with the teachings of MacDonald et al. (in which BNP was assessed in the context of canine heart failure), it would have been obvious to one of ordinary skill in the art to detect canine NT-proBNP instead of canine BNP in plasma in the method of MacDonald et al. One would be motivated to do this because Karl et al. taught that the former was known to be a more stable biomarker; as such, assessing changes in the levels of NT-proBNP in the disease state would be less subject to biomarker degradation which would be expected to adversely affect

measurements. In addition, patient samples could be stored for longer periods of time before being assayed.

In carrying out assays for canine NT-proBNP, it would have been further obvious to follow the immunoassay strategy set forth in Karl et al., which directs the skilled artisan to employ two antibodies specific for the amino-terminal fragment of pro-BNP or NT-proBNP.

As discussed above, Karl et al. suggests antibodies that recognize amino acids 10-66, 10-50, or 10-38 of the 76-residue human NT-proBNP molecule; and exemplifies antibodies that recognize amino acids 30-38.

Liu et al. teach that the nucleotide and amino acid sequences of BNP have been identified for several mammalian species, including human and dog (page 188, right column). Liu et al. teach that sequence similarity of BNP genes provides strong evidence of related function in mammalian species abstract and page 188, right column, first full paragraph). In Figure 3, the full-length sequences of preproBNP from dog, human, and other species are aligned to show similarities among species:



In their publication, Liu et al. reported the newly identified cat BNP sequence and discuss how this information now allows for antibodies to be generated using synthesized antigenic peptides for immunological assay development, and in particular for clinical applications (paragraph bridging pages 188-189).

This indicates that with knowledge of a protein's amino acid sequence, antibodies can be produced against the protein in order to detect the protein by clinical immunoassay. While the cat BNP sequence was newly reported by Liu et al., the sequence for canine preproBNP was previously known in the art (Liu et al., page 183, right column; and Figure 3). Liu et al. also teach that antibodies to BNP were known in the art to be species-specific (page 183, last column).

It is noted that the above sequences of Liu et al. are for preproBNP, and include the N-terminal signal peptide, which is 26 amino acids in length in the case of the feline, canine, and human sequences (see Liu et al., abstract).

Based on the detailed sequence information known in the prior art about the human and canine proBNP molecules (as taught by Liu et al.), one of ordinary skill in the art would have found it obvious to design and raise antibodies against canine NT-proBNP. For example, it would have been obvious to produce canine NT-proBNP in recombinant form as was done by Karl et al. for human BNP, and to raise antibodies against this molecule.

In particular, based on the teachings of Karl et al. that antibodies against residues 30-38 human proBNP can successfully be used to detect human NT-proBNP in clinical assays, one of ordinary skill in the art would have found it obvious to raise antibodies that bind within the corresponding regions of canine NT-proBNP. One would be motivated to combine the reference teachings in this manner because known work in one field of endeavor may prompt variations in another. In the instant case, one or ordinary skill in the art seeking to design antibodies to canine NT-proBNP would reasonably consider analogous art pertaining to antibodies for the human homolog.

Although the canine and human proBNP sequences do not align exactly, differing in length, the teachings of Liu et al. provide detailed information about how the amino acids in the sequence of human proBNP correspond to those in canine proBNP. Examining the sequence alignment of Liu et al. above, it can be readily seen that amino acids 30-38 of human proBNP (ELQVEQTSL; SEQ ID NO:8 in Karl et al.) correspond to amino acids 37-45 of canine proBNP (subtracting the 26-amino acid N-terminal signal sequence):

30-38 (human): ELQVEQTSL

37-45 (canine): ELQAEQLAL

This canine amino acid sequence **lies entirely within** the sequence depicted as amino acids 32-48 in the instant specification (Figure 1B).

Therefore, when producing antibodies against the canine sequence analogous to amino acids 30-38 of human proBNP, it would necessarily follow that the resulting antibodies would be specific for an epitope within amino acids 32-48 of canine proBNP.

In addition, based on the findings of Karl et al. that amino acids 30-38 of human proBNP (ELQVEQTSL) was one of two epitopes which showed the strongest reaction with the polyclonal antibodies they elicited [0084], as well as the strong sequence and structural similarity between human and canine proBNP taught by Liu et al., it would also be reasonably expected that polyclonal antibodies raised against canine NT-proBNP would also be reactive with the canine NT-proBNP epitope corresponding to amino acids 30-38 of human NT-proBNP.

As such, armed with the knowledge that amino acids 30-38 of human NT-proBNP are highly immunogenic, as well as the detailed sequence information available that these amino acids correspond to amino acids 37-45 of canine NT-proBNP and that these amino acids are largely conserved across the two species, it would have been obvious to one of ordinary skill in the art to target these analogous amino acids in canine NT-proBNP as they would have been reasonably expected to be immunogenic.

Further, when making an antibody against amino acids 37-45 of canine NT-proBNP, the resulting antibody would necessarily bind to amino acids 32-48 of this peptide since the former sequence lies entirely within the latter.

In view of the knowledge in the art of the field of human proBNP field as taught by Karl et al., and when taken together with the detailed sequence information for both human and canine proBNP available in the art (as taught by Liu et al.), the Examiner finds that the differences between the claimed invention and the prior art were encompassed in known variations.

While the above analysis focuses on the elected species of an antibody binding to an epitope within amino acids 32-48, the examiner notes that claim 29 is not limited to such an epitope but is directed to the broader range of amino acids 20-86 of proBNP. However, it is briefly noted that this broader range is also obvious in view of the reference teachings.

In particular, from the teachings of Karl et al. and Liu et al. as discussed in detail above, one would be motivated to detect canine NT-proBNP (i.e., the N-terminal portion of proBNP). The range suggested by the prior art also corresponds to the N-terminal portion of proBNP and therefore overlaps the currently claimed range, such that a *prima facie* case of obviousness exists. As such, it would also have been obvious to arrive at the claimed invention through routine optimization, by employing antibodies directed against epitopes within the N-terminal fragment of canine proBNP as suggested by Karl et al.

With respect to claim 32, Karl et al. teach that usually an epitope is clearly defined by 6 to 8 amino acids [0016]. In light of this evidence, it is presumed that the antibodies raised against canine NT-proBNP would possess the recited characteristics.

With respect to claims 33 and 58, Karl et al. teach that the antibodies may be either monoclonal or polyclonal [0025].

With respect to claims 34-36, Karl et al. teach that the antibody may be labeled for example with the hapten digoxigenin, which is then again bound by a further digoxigenin-specific antibody so as to allow for detection [0030]. This further antibody is itself labeled, for example with an enzyme such as peroxidase. Therefore, it would have been further obvious to employ such a further antibody in this manner so as to allow for detection of the results of the assay of MacDonald et al. and Karl et al.

With respect to claims 37-38, the immunoassay of Karl et al. involves binding antibody to a solid phase, such that the antigen is thereby captured on the solid phase during the assay [0026]. Such immunoassays may be characterized as an "immune binding assay" and may also be enzyme immunoassays in that they may use enzyme labels such as peroxidase [0030].

With respect to claims 59-60, McDonald et al. teaches detection of BNP in plasma as noted above. Karl et al. also teaches that all biological liquids known to the expert can be used as samples for the procedure of the method of identifying N-terminal proBNP. The samples preferred are body liquids like whole blood, blood serum, blood plasma, urine or saliva. The use of blood serum and plasma is particularly preferred. See [0031]. Therefore, it would have been further obvious to employ the methods of MacDonald et al. and Karl et al. to analyze NT-proBNP canine serum rather than plasma, given that this type of sample was identified by Karl et al. as being particularly preferred for analysis of NT-proBNP.

Double Patenting

17. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection

is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

18. Claims 29, 31-38, 47, and 57-60 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 6-12 and 21-22 of copending Application No. 12/394,731. Although the conflicting claims are not identical, they are not patentably distinct from each other because the copending application also claims a method of detecting canine proBNP or a fragment thereof in a canine sample by contacting the sample with at least one antibody that binds to an epitope within the region from **amino acids 32 to 48** of canine proBNP (see especially claims 1 and 7). The epitope may comprise at least 3 amino acids (see claims 2 and 14) and may be either monoclonal or polyclonal (claims 3-4). Detection may be via radioimmunoassay, immune binding assay, Western blot, immunohistochemistry, or enzyme immunoassay (see claim 6) and may employ peroxidase, biotin, fluorescent dye, gold colloid, or a radionuclide as labels for the antibody (see claim 16).

With respect to claim 47, although copending Application No. 12/394,731 fails to specifically recite a step of obtaining the antibody, it would have been obvious to do so as a necessary step before the antibody could be used in the recited method.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

19. Claims 29, 31-38, 47, and 57-60 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 6-12 and 21-22 of copending Application No. 12/394,682 in view of Harlow & Lane (Harlow, E. and Lane, D., Antibodies: A Laboratory Manual (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 72-77, 555-561, 578-582, and 591-592; hereafter, "Harlow & Lane **3**"), Karl et al., and Liu et al.

Copending Application No. 12/394,682 recites a method of detecting canine proBNP or a fragment thereof in a canine sample comprising (a) providing a canine blood or urine sample; (b) contacting the sample with at least one antibody that binds canine proBNP; and (c) detecting the binding of the antibody to the epitope whereby the canine proBNP or the fragment thereof in the sample is detected. See especially claim 1. The epitope may comprise at least 3 amino acids (see claims 2 and 14) and may be either monoclonal or polyclonal (claims 3-4). Detection may be via radioimmunoassay, immune binding assay, Western blot, immunohistochemistry, or enzyme immunoassay (see claim 6) and may employ peroxidase, biotin, fluorescent dye, gold colloid, or a radionuclide as labels for the antibody (see claim 16).

Copending Application No. 12/394,682 differs from the instant claims in that it recites the use of an antibody that binds an epitope in the region from amino acids 1 to 22 of canine proBNP, rather than amino acids 32 to 48 as instantly claimed.

Harlow & Lane **3** is a laboratory manual for experiments involving antibodies. Harlow & Lane **3** teach that the most useful method to detect and quantitate antigens is the **two-antibody sandwich assay**, an immunoassay that is quick, accurate and reliable (pages 555, 559 and 579). This type of assay is conducted on a test solution and requires two antibodies that bind to non-overlapping epitopes, where one of the antibodies is bound to a solid phase and the other is labeled (see especially at pages 555-561, 578-582, and 591-592). Either two monoclonal antibodies that bind to discrete sites or alternatively one batch of affinity-purified polyclonal antibodies can be used to supply these two antibodies (page 579). To make the assay quantitative, results are compared to a standard curve obtained using known amounts of pure antigen (page 582).

Harlow & Lane **3** also provide extensive guidance with regard to how to obtain antibodies that are specific for a particular region of a protein using synthetic peptides (see pages 72-77, and especially at page 73, last paragraph). Further, Harlow & Lane **3** teach that when choosing such a peptide sequence, in general peptides of approximately 10 residues should be used as the lower limit for coupling; while peptides over 20 residues in length are increasingly difficult to synthesize.

In summary, it would have been obvious to one of ordinary skill in the art to employ an additional canine proBNP antibody in the method of copending Application No. 12/394,682 in order to allow for a two-antibody sandwich assay for canine proBNP to be performed. One would be motivated to do this because Harlow & Lane **3** taught that such sandwich assay formats are the most powerful of the immunoassays.

The teachings of Karl et al. and Liu et al. are discussed in detail above. Based on the findings of Karl et al. that amino acids 30-38 of human proBNP (ELQVEQTSL) was one of two epitopes which showed the strongest reaction with the polyclonal antibodies they elicited [0084], as well as the strong sequence and structural similarity between human and canine proBNP taught by Liu et al., it would also be reasonably expected that polyclonal antibodies raised against canine NT-proBNP would also be reactive with the canine NT-proBNP epitope corresponding to amino acids 30-38 of human NT-proBNP.

As such, armed with the knowledge that amino acids 30-38 of human NT-proBNP are highly immunogenic, as well as the detailed sequence information available that these amino acids correspond to amino acids 37-45 of canine NT-proBNP and that these amino acids are largely conserved across the two species, it would have been obvious to one of ordinary skill in the art to target these analogous amino acids in canine NT-proBNP as they would have been reasonably expected to be immunogenic.

Further, when making an antibody against amino acids 37-45 of canine NT-proBNP, the resulting antibody would necessarily bind to amino acids 32-48 of this peptide since the former sequence lies entirely within the latter.

In summary, when the teachings of Karl et al. and Liu et al. are taken together with the guidance of Harlow & Lane that the two antibodies used for such assays must be *non-overlapping*, as well as the guidance that the antibodies should be raised using peptides that are generally at least 10 residues and no more than 20 residues in length, it would have been further obvious to select an antibody that bound within amino acids 32 to 48 of proBNP as claimed.

This is a provisional obviousness-type double patenting rejection.

Response to Arguments

20. Applicant's arguments filed 5/18/2010 have been fully considered.

21. Applicant's arguments with respect to the rejections under § 112, 1st paragraph (scope of enablement) have been fully considered but are not found persuasive. It is noted that Applicant's reply has necessitated additional grounds of rejection under this statute, as set forth above.

Moreover, Applicant's reply does not address all previous grounds of rejection. In particular, the preamble of claim 29 recites "[a] method of determining the presence and/or concentration of canine proBNP **or fragments thereof**". However, the claimed methods are insufficient to detect *any* fragments of canine proBNP as claimed because the epitope bound by the antibody is present within the amino-terminal portion of proBNP. Consequently, the antibody used for the assay would not bind to BNP or to other fragments that lack the SEQ ID NO:3 epitope.

Applicant's reply fails to address this aspect of the rejection, which is therefore maintained as set forth above. See also the Office action mailed 11/19/2009 at page 29, in which Applicant's failure to traverse this issue was previously noted.

22. With respect to the rejections under § 112, 2nd paragraph, Applicant's arguments (Reply, pages 9-10) have been fully considered but are not found persuasive.

Applicant argues that one could determine what is meant by amino acids 20-86 of canine proBNP by looking at the sequence published in Swiss-Prot Accession No. P16859, which is

mentioned in the instant specification. Applicant further argues that incorporation by reference is not necessary because this does not constitute "essential material".

This is not found persuasive for reasons of record (see the Office action mailed 11/29/2009 at page 30, item 24). It is maintained that because one of ordinary skill in the art would need to consult extraneous sources in order to verify the scope of the claims, such extraneous source material constitutes "essential material" per 37 CFR § 1.57 as it is necessary in order to describe the claimed invention in terms that particularly point out and distinctly claim the invention as required by the second paragraph of 35 U.S.C. 112. Such material would need to be effectively incorporated by reference into the present application, which has not been done.

Moreover, while Applicant points to the database sequence recited in P16859 as being the sequence of proBNP, the Office has repeatedly noted that there are discrepancies between this sequence and those sequences disclosed in the instant specification. In other words, amino acids 20-86 of canine proBNP do not appear to correspond to amino acids of 20-86 of the sequence currently listed under the database entry P16859. Applicant's reply does not address this issue.

23. With respect to the rejections under § 103 based upon MacDonald et al. in view of Asada et al., Applicant's arguments (Reply, pages 9-16) have been fully considered but are not found persuasive. Applicant also relies upon the accompanying Declarations by Drs. Farace and Boswood.

Applicant's arguments, as well as the Declarations under 37 CFR 1.132 filed 5/24/2010 are insufficient to overcome the rejections based upon 35 U.S.C. 103(a) as set forth in the last Office action for the following reasons.

Applicant argues for unpredictability and uncertainty surrounding BNP at the time of the instant invention. Specifically, Applicant argues that although Asada reports that human BNP exists primarily in human blood as proBNP rather than BNP-32, the reference does not does not disclose which form of canine BNP is predominant in canine blood. Applicant argues that the Office merely speculates that such findings would apply to canines. Applicant points to the Boswood Declaration and argues that it was not possible to predict that one could detect canine proBNP in blood or urine based on teachings regarding detection of human BNP. See Reply, pages 10-11, and the Boswood Declaration at items 19-20 in particular.

This is not found persuasive because Asada clearly contemplates any mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1). Asada teach that mammalian proBNP, a term which they indicate includes canine proBNP, can be detected in blood (ibid). Moreover, the statements made by Asada that in blood, BNP exists in the form of proBNP or its degradation product, and not in the form of BNP-32 which was previously considered dominant [0008], are made generally and are not qualified as referring only to human BNP as apparently perceived by Applicant.

The evidence of record therefore fails to support the testimony of Dr. Boswood that it was unknown whether circulating proBNP could be detected in dogs. As such, it is maintained that the ordinary artisan would reasonably expect success in detecting canine proBNP in blood, even absent an actual reduction of practice in the prior art.

Further, from the observation of canine BNP in blood (as taught by MacDonald), the existence of the larger proBNP precursor may be inferred. The existence of the mature peptide is strong evidence of the existence of its precursor. Similarly, as both the precursor proBNP as well

as the C-terminal fragment thereof (BNP) were known to exist in dogs, it would have been reasonably expected that an N-terminal fragment of proBNP would also exist.

Consequently, the evidence indicates that one skill in the art would have had a reasonable expectation of success in detecting not only circulating proBNP (as taught by Asada et al.) or the circulating C-terminal fragment thereof (as taught by MacDonald et al.) but also an N-terminal fragment thereof containing amino acids 32-48.

Applicant further argues that uncertainty persisted regarding the particular forms of proBNP/BNP that existed in humans, and that in canines even less was known (Reply, pages 12-15). In particular, Applicant argues that it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP. For example, Applicant argues that it was unpredictable whether a particular region of canine proBNP would be accessible for antibody binding. See Reply, pages 12-13, and the Boswood Declaration at items 3, 5, and 7-15 in particular.

Initially, it is noted that the claims recite a method of detecting "canine proBNP or fragments thereof", and therefore do not require detection of any one "particular" fragment of proBNP. Rather, the claims invoke detection of either canine proBNP or fragment thereof in the alternative. As such, Applicant's arguments that it was not possible to predict that an antibody could detect "a particular N-terminal fragment of proBNP" are not on point as the claims do not require this.

Applicant is also reminded that obviousness does not require absolute predictability of success. Indeed, for many inventions that seem quite obvious, there is no absolute predictability

of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious. In re Merck & Co., 800 F.2d at 1098, 231 USPQ at 380; Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 1461, 221 USPQ 481, 488 (Fed.Cir.1984); In re Papesch, 315 F.2d 381, 386-87, 137 USPQ 43, 47-48 (CCPA 1963). For obviousness under § 103, all that is required is a reasonable expectation of success. In re Longi, 759 F.2d 887, 897, 225 USPQ 645, 651-52 (Fed. Cir. 1985); In re Clinton, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976).

In the instant case, it was known that antibodies could be raised against almost any substance. For example, Janeway et al. (Immunobiology: the Immune System in Health and Disease (1999), Elsevier Science Ltd/Garland Publishing, New York, NY, Fourth Edition, page 36) taught that "any structure can be recognized by antibody as an antigen" (see page 36, first full paragraph). Therefore, it could have been *reasonably* expected that antibodies could be made against amino acids 32 to 48 of canine proBNP.

Furthermore, in the instant case additional specific facts were known in the prior art that would lead one of ordinary skill in the art to reasonably expect success in raising antibodies against amino acids 32 to 48 of canine proBNP.

Specifically, the amino acid sequences of both human and canine proBNP were known in the prior art. Liu et al. (discussed in detail above) taught there are "highly conserved regions" in BNP sequences across species, including between human and dog preproBNP. See section 3.3 and Figure 3.

In the instant case, amino acids 37-45 of canine proBNP were known to share significant sequence identity with a corresponding region in human proBNP known to be highly immunogenic. As such, it is maintained that the ordinary artisan would have had a reasonable expectation of success in designing antibodies that bind within the claimed range.

Moreover, although the amino acid sequences of BNPs across species may differ, at the time of the invention the sequence of canine BNP was known in the art. It was also well known in the art how to raise antibodies against a known amino acid sequence.

As illustration of this common knowledge in the art, Luchner et al. (Am J Physiol Heart Circ Physiol 274:1684-1689, 1998, Applicant's Information Disclosure Statement of 6/3/2010) concisely state how they employed a polyclonal antibody specific for canine BNP in order to measure BNP in dogs, because of the known species variability in BNP (see page 1685, "Analytical methods"). This provides evidence that species-specific differences were known but that rather than presenting an insurmountable technical obstacle, the ordinary artisan was familiar with how to deal with this issue; namely by using species-specific antibodies.

Similarly, the evidence of record indicates that notwithstanding the known differences across species, BNP was nonetheless thought to share related functions in mammalian species. See for example Liu et al. at page 188, right column, first full paragraph, last sentence.

For all of these reasons, the opinion testimony of Dr. Boswood fails to constitute sufficient evidence that one of ordinary skill in the art would be unable to predict that an antibody against amino acids 32 to 48 of canine proBNP could be made.

Applicant also argues that it was also unknown and could not be predicted how many forms of BNP circulate in canine blood or urine, and whether such forms were stable enough to be detected by antibodies. See Reply, pages 13-14, and the Declaration of Dr. Boswood at items 7-8, 14, and 19-20 in particular. The Declaration of Dr. Boswood goes so far as to argue that "it was unknown whether circulating proBNP could be detected in dogs or cats" (item 20).

This is not found persuasive because initially, the claimed methods employ an antibody directed against amino acids 32-48 of canine proBNP. Performance of such methods do not require knowledge of how many forms of BNP are present, of the identities of such forms, or of which forms predominate. As noted above the claimed methods do not require detection of any *particular* form of BNP, but rather encompass detection of proBNP or (any) fragment thereof in the alternative. As such, knowledge of "how many forms of BNP circulate" is not required to practice the claimed invention. Consequently, a determination of obviousness only requires a reasonable expectation of success in detecting BNP molecules in canine blood that include this region of proBNP. Even if one of ordinary skill in the art would have also expected canine BNP to exhibit heterogeneity, this general suggestion does not constitute sufficient evidence of unpredictability in detecting forms that include amino acids 32-48 of proBNP.

In this regard, it is noted that the claimed region resides within the amino terminus of proBNP. It is maintained that it was well known in the art at the time of the invention that proBNP is the prohormone precursor of BNP, which is a C-terminal fragment of proBNP released by proteolytic cleavage of the larger proBNP molecule. This is discussed for example by Asada et al. at [0002]. Further, as discussed above, Asada et al. taught detection of

mammalian proBNP in blood, including canine proBNP (see [0010] and claim 1). There is nothing of record that would clearly cast doubt on this teaching.

In view of these teachings, it is maintained that the ordinary artisan would reasonably expect success in detecting canine proBNP in blood, even absent an actual reduction of practice in the prior art. As above, the evidence of record fails to support the testimony of Dr. Boswood that it was unknown whether circulating proBNP could be detected in dogs. Similarly, the evidence of Asada et al. contradicts the arguments of counsel that "no cited reference discloses any teachings regarding proBNP in canines" (see Reply, page 13, first full paragraph).

Further, the prior art also recognized that the mature form of BNP is present in canine blood (as taught by MacDonald). The existence of the mature BNP peptide is strong evidence of the existence of its longer proBNP precursor, since mature BNP was known to be produced by proteolytic cleavage of the proBNP precursor.

Consequently, the evidence indicates that one skill in the art would have had a reasonable expectation of success in detecting not only circulating proBNP (as taught by Asada et al.) or the circulating C-terminal fragment thereof (as taught by MacDonald et al.) but also proBNP fragments containing amino acids 32-48.

Applicant further points to the teachings of Goetze et al. as providing evidence of uncertainty (Reply, pages 12-13, section **a)**; Declaration of Dr. Boswood at items 4-7). In particular, Applicant points to statements made by Goetze et al. that oligomerization of proBNP may have a major influence on assays. Applicant argues that it is not possible to predict how many forms of BNP were present in canine blood and which would predominant.

Applicant's reliance on Goetze et al., which pertains to human proBNP, is logically inconsistent with Applicant's position taken elsewhere that studies regarding human proBNP cannot be translated to canine proBNP (Reply, page 12; Declaration of Dr. Boswood, item 15). In particular, Dr. Boswood declares that "based on the discovery of a particular scientific phenomenon regarding BNP in one species (e.g., human), one could not reasonably predict that the same phenomenon will exist in a different species (e.g., dog or cat)". Declaration of Dr. Boswood at item 15.

If one adopts this position of Dr. Boswood, then the ordinary artisan would not draw any conclusions about canine BNP from Goetze et al.'s observation of oligomerization in human proBNP.

In addition, the fact that human proBNP molecules may oligomerize does not necessarily mean that detection of canine proBNP molecules was unpredictable. In particular, there is abundant evidence of record available that despite any such oligomerization activity, human proBNP and NT-proBNP was nonetheless routinely measured (Karl et al., Asada et al., Davey et al. (U.S. 2004/0096920). As such, the general mention of possible oligomerization does not provide evidence of a substantial technical obstacle.

Applicant further argues that the half-life of canine BNP was known to be shorter than the half-life of BNP in other species (Declaration of Dr. Boswood, item 16). However, whether the ordinary artisan would expect different BNP forms to have the same half-lives in different species is tangential, as the claimed invention relates not to the assay of BNP but of proBNP or fragments thereof. In the instant case, it was known that one role of the N-terminal structure of

proBNP was stabilization (Asada et al., column 2, lines 28-41; Karl et al., [0006]-[0007]). Moreover, Karl discusses the stability of BNP in general and does not qualify these remarks in terms of *human* BNP alone. Rather, in one passage in which Karl et al. discuss the stability of BNP, the reference explicitly mentions both human and pig BNP [0006]. Consequently, it is maintained that one of ordinary skill in the art would *reasonably* expect the mature form of BNP to be less stable across species.

As such, it would be reasonable to expect that within a given species, proBNP or fragments thereof (such as NT-proBNP) would have longer half-lives than the mature BNP form; regardless of whether the precise half-lives were numerically the same across species. Consequently, the noted information is not found to represent persuasive evidence of non-obviousness.

Applicant also points to results obtained when canine BNP was used as a therapeutic agent, arguing that because the effects of canine BNP persisted longer that this is evidence that BNP signaling in dogs is distinct from BNP signaling in other species (Declaration of Dr. Boswood, item 16).

This is not found persuasive because such information about the use of canine BNP as a therapeutic is much less informative in regards to the claimed assay methods than the direct evidence already of record, which indicate that BNP was known to be correlated with heart disease both in dogs and in humans. Whether the effects of exogenously administered BNP differ across species fails to detract from this highly relevant available knowledge.

Similarly, observations regarding species differences in regards to the pattern of BNP concentration in relation to age (Declaration of Dr. Boswood, item 17) are seen as tangential to

the claimed assay method, which do not require measurement of proBNP as a function of age. The examiner disagrees that such knowledge would cast doubt on the existing knowledge in the art that natriuretic peptides can serve as markers of heart disease across species.

Similarly, Applicant relies upon Luchner et al. as evidence that BNP molecules may not be useful indicators of heart failure in all species (Declaration of Dr. Boswood, item 18). However, the passage of Luchner indicated by Applicant says merely that the activation of the cardiac natriuretic peptide system may be different across species in that it is activated later in dogs. This is different from saying that BNP is not expected to be a marker of heart failure at all.

Consequently, the fact that human and canine sequences differ does not constitute evidence of non-obviousness, as the ordinary artisan would have been familiar with this type of issue and would have known how to raise antibodies against canine proBNP as its amino acid sequence was known. As such, Applicant's emphasis on the species-specific sequence differences does not provide evidence of unpredictability in raising antibodies against canine proBNP. Similarly, the fact that BNP molecules may have different properties in different species fails to detract from the available knowledge in the art that the BNP system was known to be activated in the context of heart disease in both humans and in dogs.

The Declaration of Dr. Farace reports the results of experiments showing that human and canine NTproBNPs are structurally different and antibodies are species specific (see also the instant reply, paragraph bridging pages 14-15). Such evidence has been considered but as above, is not persuasive evidence of non-obviousness because as discussed above, one of ordinary skill in the art would have known how to raise antibodies specific for canine NTproBNP.

Notwithstanding the above, it is noted that the experiments reported in the Declaration do not clearly support the conclusion that the antibodies involved in these studies are species-specific.

In particular, the experiment involved the use of **two** antibodies in order to form a sandwich complex. This type of experimental design therefore requires that **both** of the antibodies cross-react with NTproBNP of another species. The data obtained from this type of experiment would only allow one to conclude that one or both of the antibodies is species-specific. However, it cannot be ruled out that one of the antibodies would cross-react among different species.

Indeed, the data presented in the instant specification clearly indicate that an antibody specific to amino acids 1-22 of canine NT-proBNP does in fact cross-react with human NT-proBNP (see Table 4, first line).

The evidence of the specification therefore indicates that canine proBNP is in fact structurally related to human proBNP. For all of these reasons, the evidence of Dr. Farace is insufficient to establish that canine and human proBNP are so structurally different that one of ordinary skill in the art would lack a reasonable expectation of success in translating the available knowledge regarding human proBNP to the assay of canine proBNP.

Applicant also argues that it was unclear which of the various BNP forms would be useful as **markers of pathophysiologic processes** (Declaration of Dr. Boswood, items 7-8).

Initially, it is noted that the claimed invention is directed only to immunoassay methods and not to methods of using particular BNP forms as disease markers. Moreover, in support of

this position Applicant is relying on statements made by Goetze in regards to human BNP. As discussed above, this reliance is inconsistent with the position taken elsewhere by Applicant that facts about human BNP cannot be extrapolated to other species. In addition, Even if one of ordinary skill in the art would have also expected canine BNP to exhibit heterogeneity, this general suggestion does not constitute sufficient evidence of unpredictability in detecting forms that include the amino terminus of proBNP.

Applicant also analyzes the Goetze et al. reference as indicating that it was unclear which of the various forms would be useful as markers (Declaration of Dr. Boswood at item 7). However, in the passage which Applicant cites at item 7 of the Declaration, Goetze et al. merely state that different products may not always be "equal" markers of the same pathophysiological processes. This is not the same as suggesting that some forms may be useful markers and others may not.

Applicant does not separately argue the limitations of dependent claims 34-36 (see Reply, pages 16-17).

For all of these reasons, the evidence of non-obviousness fails to outweigh the evidence of obviousness.

24. With respect to the rejections under § 103 based upon MacDonald et al. in view of Karl et al. and Liu et al., Applicant's arguments (Reply, page 17) have been fully considered but are not found persuasive.

Applicant points to the Declaration of Dr. Boswood and argues that it was not previously known which fragments of canine proBNP circulated in blood or whether any such fragments were sufficiently abundant and stable to be detected by immunoassay.

Applicant further argues that while Karl reported that human BNP is more stable than BNP-32, the reference does not discuss the stability of canine BNP. As best understood, Applicant here means to contrast NT-proBNP with BNP-32 (and not BNP with BNP-32).

This is not found persuasive because it was known that one role of the N-terminal structure of proBNP was stabilization (Asada et al., [0008]; Karl et al., [0006]-[0007]). Moreover, Karl discusses the stability of mature BNP in general and does not qualify these remarks in terms of *human* BNP. Rather, in one passage in which Karl et al. discuss the stability of BNP, the reference explicitly mentions both human and pig BNP [0006]. Consequently, it is maintained that one of ordinary skill in the art would *reasonably* expect the mature form of BNP to be less stable in canines as well as in humans.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5:00. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya, can be reached at (571) 272-0806. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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